Observations on Midgut Structure and Content of *Chrysoperla carnea* (Neuroptera: Chrysopidae)

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ABSTRACT A study of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) midgut structures and contents was conducted using scanning and transmission electron microscopy. The larval midgut was enclosed by a peritrophic membrane that seemed to be composed of two layers. Numerous bacteria were found throughout the lumen of the midgut, and because the midgut does not open to the hindgut, we hypothesized that the bacteria may serve to decompose the residues occurring in the midgut lumen. Few yeast cells were present in the larval midgut. The visual observations suggest that the concentration of bacteria were much lower in adult midguts than in larval midguts. However, many yeast cells were observed in the lumen of the adult midgut. No obvious peritrophic membrane was observed in electron micrographs of the adult midgut compared with the larval midgut. The results suggest different modes of food residue disposal.

KEY WORDS *Chrysoperla carnea*, midgut, bacteria, yeast, larvae

Green lacewings (Neuroptera: Chrysopidae) are important biological control agents (Canard et al. 1984, Tauber et al. 2000). *Chrysoperla carnea* (Stephens) and *Chrysoperla rufilabris* (Burmeister) are the most commonly used species in biological control programs in North America and Europe (Tauber et al. 2000). The development of new, high-quality, cost-effective artificial diets can reduce current rearing costs and increase the availability and reliability of mass-reared *Chrysoperla* spp. Knowledge of the feeding habitats and characteristics of digestive systems will support the development of artificial diets (Hagen et al. 1970).

All chrysopid larvae are characteristically voracious, and they often have a broad prey range. They feed on small, comparatively soft-bodied arthropods, including aphids, leafhoppers, whiteflies, psyllids (Homoptera); thrips (Thysanoptera); and eggs of numerous pest insect species. Lepidoptera larvae also are attacked (Principi and Canard 1984). The morphology of chrysopid larvae alimentary canals has been studied in several species, including *Chrysopa perla* (L.), *Chrysopa oculata* Say, *Chrysopa cubana* (Hagen), and *Chrysopa* (=*Chrysoperla*) *carnea* (Stephens). The alimentary canals of all the species studied are closed between the mid- and hindgut (McDunnough 1909, Spiegler 1962, Jepp 1984). Therefore, we hypothesized that microbes might be present to decompose the digestive residues in the midgut.

Some adult chryopids feed as carnivores, as do the larvae, and are also glyco-pollinivores, which is the use of insect honeydew; and they also may feed on pollen and flower nectar (Principi and Canard 1984). Adults of the genus *Chrysoperla* are glyco-pollinivorous. Yeast symbionts have been identified in the alimentary canals of some glyco-pollinivourors adults. Hagen et al. (1970) observed large numbers of yeast cells, belonging to the genus *Torulopsis*, within the crop diverticulum of *C. carnea.* Woolfolk and Iglis (2003) and Woolfolk et al. (2004) found that yeast cells (primarily *Metschnikowia pulcherrima* Pitt and Miller) existed throughout the alimentary canal of *C. rufilabris* adults, specifically in the crop diverticulae, fore, mid-, and hindguts. The existence of yeast cells in the midguts of *C. carnea* has not been documented. Additionally, the existence of the peritrophic membrane in Chrysopidae has been questioned (Bitsch 1984). Thus, we used electron microscopy to improve our knowledge of the midgut structures and contents of *C. carnea.*

Materials and Methods

Rearing of *C. carnea.* Eggs and pupae of *C. carnea* were obtained from Ricon-Vitova Insectaries Inc. (Ventura, CA). Newly hatched larvae were reared individually in clear plastic petri dishes (5.5 cm in diameter) and fed with cotton aphids, *Aphis gossypii* Glover (Homoptera: Aphididae), on cotton *Gossypium hirsutum* L. 'Deltapine 5415' leaves. Newly emerged adults were reared in plastic cages (28 cm in diameter and 62 cm in length) containing potted cotton plants infested with *A. gossypii.* The *C. carnea* adults fed on the aphid honeydew. The rearing con-

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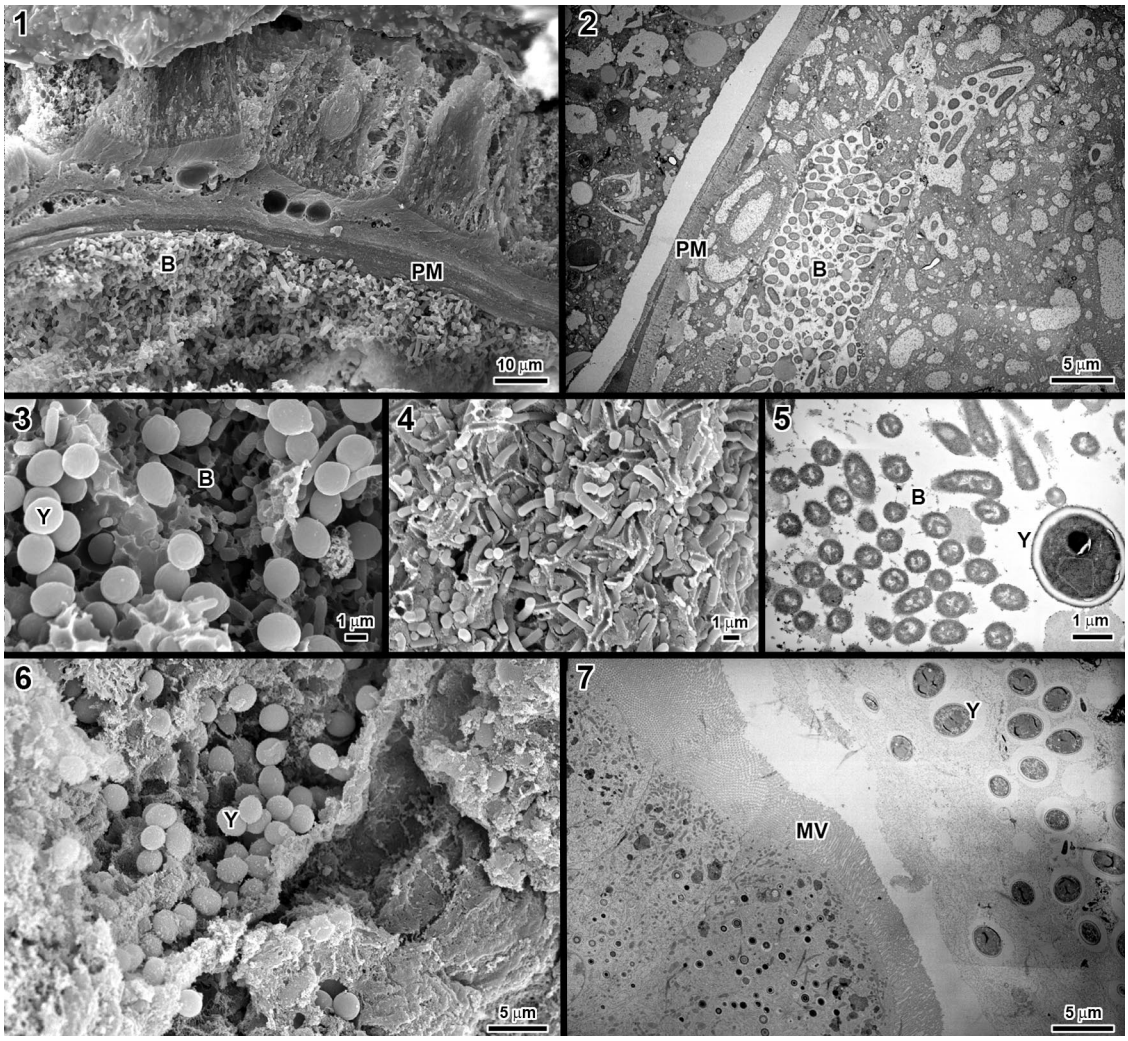

Fig. 1. Electron micrographs. (1) SEM of a *C. carnea* larval midgut, showing numerous bacteria (B) and a peritrophic membrane (PM) within the lumen. (2) SEM of a *C. carnea* larval midgut, showing bacteria (B) and peritrophic membrane (PM) within the lumen. (3) SEM of a *C. carnea* larval midgut, showing bacteria (B) and yeast (Y) within the lumen. (4) TEM of a *C. carnea* larval midgut, showing numerous bacteria within the midgut lumen (higher magnification). (5) TEM of a *C. carnea* larval midgut, showing bacteria (B) and yeast (Y) within the lumen. (6) SEM of a *C. carnea* adult midgut, showing yeast (Y) within the lumen. (7) TEM of a *C. carnea* adult midgut, showing microvilli (MV) and yeast (Y) within the lumen.

ditions were 22–25°C, 30–40% RH, and a photoperiod of 16:8 (L:D) h. Third instars and 7-d-old adults were used in the microscopy studies.

Scanning Electron Microscopy (SEM). To obtain the midguts, 12 third instar and 12 adult *C. carnea* were dissected under a stereomicroscope. The midguts were prefixed in 2.5% glutaraldehyde in Millonig's buffer, rinsed in buffer, postfixed in 2% osmium tetroxide in buffer, rinsed in distilled water, dehydrated in a graded ethanol series, cryofractured in absolute ethanol, critical point dried, sputter coated with gold or gold palladium, and examined with a JEOL 6400 SEM (JEOL, Tokyo, Japan) at 15 kV.

Transmission Electron Microscopy (TEM). The midguts were prefixed in 2.5% buffered glutaraldehyde, postfixed in 2% buffered osmium tetroxide, dehydrated in a graded acetone series, and embedded in Epon-Araldite's epoxy resin. The sections were cut with an ultramicrotome, stained with uranyl acetate and lead citrate, and observed with a JEOL 100CX TEM at 60 kV.

Results and Discussion

Midguts of *C. carnea* Larvae. Dense bacterial populations were observed throughout the midgut lumen of larval *C. carnea* (Fig. 1, 1–5). The function of the bacteria is unknown. However, because the midgut does not open to the hindgut, the bacteria may serve to decompose residues occurring in the midgut lumen.

Many insect species are known to have symbiotic relationships with microorganisms that occur in their alimentary canals. Some symbiont species are contained in specialized mycetocyte cells, whereas in species, the microorganisms are free in the gut lumen (Chapman 1985). For *C. carnea* larvae, as shown in Fig. 1 (1–5), the bacteria are free in the lumen. Some yeast cells also were found in the midgut lumen (Fig. 1, 1, 3, and 5), but in densities much lower than for bacteria.

Peritrophic Membrane Present in Midgut of *C. carnea* Larvae. The membrane seems to have two layers (Fig. 1, 1 and 2). In some Orthoptera, peritrophic membranes have several similar layers, whereas the dipteran *Aedes aegypti* (L.) (Culicidae) has a peritrophic membrane that is composed several structurally different layers (Chapman 1985). Two major functions of the midgut peritrophic membrane have been reported: protecting the midgut cells from abrasion by food particles and providing a barrier against certain microorganisms (Chapman 1985). These functions may be related to the double layers that were identified. However, the role of the peritrophic membrane in *C. carnea* larvae may be for enclosing the bacteria in the lumen, rather than for protecting midgut cells from abrasion by food particles, because *C. carnea* larvae siphon the fluid contents from their prey.

Midguts of Adult *C. carnea*. Large numbers of yeast cells were observed in the midgut lumen of adult *C. carnea* (Fig. 1, 6, and 7). Hagen et al. (1970) concluded that the yeast symbionts in the crop deverticulae of *C. carnea* provided the amino acid valine to adults. Woolfolk and Iglis (2003) and Woolfolk et al. (2004) postulated that yeast cells observed in the midgut of *C. rufilabris* served as a nutrient source, which also may be the case for *C. carnea.*

Fewer bacteria were found in the midgut lumens of adult *C. carnea* compared with the larval midguts. Bacteria also were found in the alimentary canal of adult *C. rufilabris* (Woolfolk and Iglis 2003, Woolfolk et al. 2004), and their data indicate that bacteria are transient and not true residents of the alimentary canals.

In our studies, no obvious peritrophic membrane was observed in the midguts of adult *C. carnea* (Fig. 1, 1 and 7). In contrast, a well developed peritrophic membrane was found in the midguts of adult *C. rufilabris* (Woolfolk et al. 2004). Chapman (1985) pointed out that in some cases it is possible that the failure to observe the membrane has resulted from examining the insects at an inappropriate time during the developmental stage of the species. The precise time the peritrophic membrane is formed in adults is unknown. Our examination of young adult midgut may account for the absence of peritrophic membranes.

Our finding of dense bacteria populations in the midgut of larval *C. carnea* may help in developing new artificial diets. Although the functions of the bacteria have not determined, their natural existence could be beneficial to the growth and development of larval *C. carnea.* Therefore, some antibiotic materials may need to be excluded from the artificial diets, and some ingredients, which are favorable to the bacteria, may need to be added. We are identifying bacterial species in green lacewing larval lumens for any potential use of the bacteria in insect pest management.

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